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Intended use of Reference Products & WHO International Standards/Reference Reagents in the development of Similar Biological Products (Biosimilars)

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A B S T R A C T

Reference Products and WHO International Standards/Reference Reagents have roles to play in the development and characterization of similar biological products (SBPs). However, these roles are distinct and non-interchangeable. The uses of these materials and their limitations are considered in this paper.

1. Introduction

Both reference products and WHO International standards/reference reagents have distinct and important roles to play in the development and characterization of similar biological products. In this article, the intended purpose of these materials is described.

2. Uses of the Reference Product

The reference product is key and fundamental in the development of SBPs. It is the 'comparator' for all the comparability studies i.e. for quality, nonclinical and clinical assessment [1–4]. It is ideally a product that has been approved and marketed in the relevant country or geographical area, which has a long established history of good efficacy and safety. It should be marketed at a level which allows the purchase of a number of different batches, so that the comparability assessment can be sufficiently thorough. This implies that the most likely reference product for use as a comparator in SBP development will be the market leader in the country where the SBP is being developed, although this is not a requirement.

For quality assessment, the SBP should be compared directly in the same assays (i.e. a head-to-head comparison) with the reference product using a range of biological, physico-chemical and sometimes immunological procedures (Table 1). The choice of such methods depends on the biosimilar under development. In some cases, the reference product can be used directly as purchased e.g., from a pharmacy, but in other cases, 'extraction' of the drug substance from the product is needed as the excipients used in the formulation may interfere with at least some procedures used for analysis. In such instances, it is important to avoid changes induced in the product by the extraction method. In some situations where this problem is likely to be encountered, it may be advantageous to also treat the SBP with the same extraction procedure used for the reference product to attempt to replicate the effects induced.

For the nonclinical and clinical studies, the SBP must be compared to the reference product directly (using the head-to-head, comparative approach). Clinical trials need to be carefully designed to assess comparative efficacy and safety.

The same reference product should be used throughout the quality, safety and efficacy comparability program during the development of a biosimilar product. The dosage form, dose, and route of administration of the biosimilar biological product should be identical to those of the reference product. A product authorized as a biosimilar product should not be used as the reference product.

3. Uses of WHO International Standards/Reference Reagents

WHO International Standards/Reference Reagents are primary standards (sometimes referred to as 'gold' standards) and are available for a wide range of substances (see Table 2). They are produced to defined standards [5] which optimize retention of biological activity and other important characteristics as well as ensuring stability. These reagents are established by the Expert Committee on Biological Standardization of WHO following an international collaborative study in which the suitability of the preparation to serve as a standard is demonstrated (for example, see [6]). They are intended to last for a considerable period, (usually several decades) as replacing them is time consuming and expensive and they often contain excipients which may interfere with physico-chemical methods. Such standards are produced in

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Table 1
Examples of analytical tests used for characterization of biotherapeutic medicines.

Parameter	Analytical test
Primary structure	Amino acid analysis, LC-ESI-MS, N/C-terminal sequencing, peptide map, MS, RP-HPLC
Higher order structure	CD spectroscopy, NMR spectroscopy, peptide map, SE-HPLC, SDS-PAGE, bioassay, binding assay
Size	AUC, MALDI TOF MS, LC-ESI-MS, SE-HPLC, SDS-PAGE
Charge	IEC, IEF, CE
Glycosylation pattern/ sequence, glycosylation site	CE, LC-ESI-MS, MALDI TOF MS, RP-HPLC, peptide map, HPAEC, IEC-HPLC
Heterogeneity e.g., Amino acid sequence/ substitution, deamidation, oxidation, aggregation, truncation/fragmentation	CE, Peptide map, RP-HPLC, SE-HPLC, IEC-HPLC, SDS-PAGE, MS, IEF, AUC, DLS, CE

LC – liquid chromatography, ESI – electrospray ionization, MS – mass spectrometry, CD – circular dichroism, NMR – nuclear magnetic resonance, AUC – analytical ultracentrifugation, SE-HPLC – Size exclusion high performance liquid chromatography, RP-HPLC – reverse phase high performance liquid chromatography, IEC – Ion exchange chromatography, IEF – Isoelectric focusing, CE – Capillary electrophoresis, SDS-PAGE – Sodium dodecyl sulfate polyacrylamide gel electrophoresis, DLS – dynamic light scattering, MALDI – TOF MS, matrix assisted laser desorption/ ionization time of flight, HPAEC – high pH anion exchange chromatography.

relatively small numbers (normally a few thousand ampoules) and are used either to calibrate secondary standards, such European Pharmacopoeial or USP Reference materials, or manufacturers working standards.

Although primarily intended for the calibration, characterization and validation of potency assays, often bioassays [7], the substances can also be used with other assays and procedures in some cases. Such standards are usually calibrated in units or International Units which are often arbitrarily defined and relate to the ampoule content of the analyte. These standards are used to calibrate bioassays either directly or for calibration of secondary or working standards [6,7]. The use of a single primary standard worldwide facilitates the comparability of assay results. Standards must be included in each bioassay run and the potency of unknown samples can be calculated by comparison of the displacement of their dose–response curves from that generated using the standard. Calibration of bioassays in terms of parameters such as 50% maximum proliferation etc. is not normally valid as this effectively ignores the potency of the standard.

Bioassay-derived potency data must be accompanied by a valid statement of the uncertainty associated with the measurement [7]. Statistical procedures have been devised for the calculation of the degree of error associated with such determinations. This is

Table 2
Available WHO International Standards, WHO Reference Reagents and NIBSC Reference Reagents for Biotherapeutics. A list of WHO International Standards is available on the NIBSC (http://www.nibsc.ac.uk/products/biological_reference_materials/product_catalogue.aspx) and WHO websites (http://www.who.int/bloodproducts/catalogue/CytoMarch11.pdf).

International Standards/Reference Reagents	Code
Alpha-1-antitrypsin (WHO 1st International Standard)	05/162
Antithrombin, concentrate, human (WHO 3rd International Standard)	06/166
Factors II & X, concentrate, human (WHO 3rd International Standard)	98/590
Factor VII, concentrate, human (WHO 1st International Standard)	97/592
Factor VIIa, concentrate (WHO 2nd International Standard) (07/228)	07/228
Factor VIII, concentrate, human (WHO 8th International Standard)	07/350
Factor IX, concentrate, human (WHO 4th International Standard)	07/182
Factor IXa, concentrate, human (WHO 1st International Standard)	97/562
Factor XIII, plasma, human (WHO 1st International Standard)	02/206
Factor Eight Inhibitor By-passing Activity (FEIBA), concentrate (1st NIBSC Working Standard)	06/172
Fibrinogen, concentrate, human (WHO 1st International Standard)	98/614
Heparin, Low Molecular Weight (WHO 2nd International Standard)	01/608[a]
Heparin, Unfractionated, porcine (WHO 6th International Standard)	07/328
Prekallikrein activator, human (WHO 2nd International Standard)	02/168
Protein C, concentrate, human (WHO 1st International Standard)	04/252
Staphylokinase (NIBSC Reagent)	94/718
Streptodornase (WHO 2nd International Standard)	08/230
Streptokinase (WHO 3rd International Standard)	00/464
Thrombin (WHO 2nd International Standard)	01/580
Urokinase, high molecular weight (WHO 1st International Standard)	87/594
von Willebrand factor, concentrate, human (WHO 1st International Standard)	00/514
Activin A, Human, Recombinant (1st WHO International Standard)	91/626
C-Peptide of Human Insulin (1st International Reference Reagent)	84/510
Calcitonin, ASU 1–7 Eel Calcitonin Analog (Elcatonin) (1st WHO International Standard)	84/614
Calcitonin, Eel (1st WHO International Standard)	88/556
Calcitonin, Human (2nd WHO International Standard)	89/620
Calcitonin, Salmon (3rd WHO International Standard)	98/586
Chorionic Gonadatrophin, beta core fragment, Human, for immunoassay (1st WHO Reference Reagent)	99/708
Chorionic Gonadatrophin, intact, Human, for immunoassay (1st WHO Reference Reagent)	99/688
Chorionic Gonadotrophin alpha subunit, Human (1st International Reference Preparation)	75/569
Chorionic Gonadotrophin beta Subunit, Human (1st International Reference Preparation)	75/551
Chorionic Gonadotrophin, alpha subunit, Human (purified), for immunoassay (1st WHO Reference Reagent)	99/720
Chorionic Gonadotrophin, beta subunit, Human, for immunoassay (1st WHO Reference Reagent)	99/720
Chorionic Gonadotrophin (5th WHO International Standard)	07/364
Chorionic Gonadotrophin, nicked beta subunit, Human, for immunoassay (1st WHO Reference Reagent)	99/692
Chorionic Gonadotrophin, nicked, human, for immunoassay (1st WHO Reference Reagent)	99/642
Corticotrophin (ACTH), Human (NIBSC Research Reagent)	74/555
Erythropoietin, Human Recombinant (2nd WHO International Standard)	88/574[a]
Follicle Stimulating Hormone and Luteinizing Hormone, Human Urinary, for bioassay (4th WHO International Standard)	98/704[a]
Follicle Stimulating Hormone, Human, Recombinant for immunoassay (1st WHO International Standard)	92/510
Follicle Stimulating Hormone, Human, Recombinant for bioassay (1st WHO International Standard)	92/642[a]
Follicle Stimulating Hormone, Pituitary (1st WHO International Standard)	83/575

(*continued on next page*)

Table 2 (*continued*)

International Standards/Reference Reagents	Code
Follicle Stimulating Hormone, urofollitropin, Human Urinary for bioassay (1st WHO International Standard)	92/512
Glucagon, Porcine (1st WHO International Standard)	69/194
Growth Hormone, Human, Pituitary (1st WHO International Standard)	80/505[a]
Inhibin A, Human, recombinant (1st WHO International Standard)	91/624
Inhibin B, Human (1st WHO Reference Reagent)	96/784
Inhibin, Porcine (1st WHO International Standard)	86/690
Insulin Like Growth Factor- I (1st WHO International Standard)	02/254
Insulin, Bovine (1st WHO International Standard)	83/511
Insulin, Human (1st WHO International Standard)	83/500[a]
Insulin, Human for immunoassay (1st International Reference Preparation)	66/304
Insulin, Porcine (1st WHO International Standard)	83/515
Insulin-Like Growth Factor Binding Protein-3 (NIBSC Research Reagent	93/560
Insulin-like growth factor-1 for bioassay (1st WHO International Standard)	91/554
Insulin-like growth factor-II, Human, recombinant (1st WHO Reference Reagent)	96/538
Luteinizing Hormone, Human, recombinant (1st WHO International Standard)	96/602[a]
Luteinizing Hormone, Human, Pituitary (2nd WHO International Standard)	80/552
Luteinizing Hormone, Pituitary, alpha subunit (1st WHO International Standard)	78/554
Luteinizing Hormone, Pituitary, beta subunit (1st WHO International Standard)	78/556
Parathyroid hormone 1-34 Recombinant, Human (1st WHO International Standard)	04/200
Parathyroid Hormone, Bovine, for bioassay (1st WHO International Standard)	82/632
Parathyroid Hormone, Human (NIBSC Research Standard)	75/549
Parathyroid Hormone, Human, for immunoassay (1st International Reference Preparation)	79/500
Parathyroid Hormone, Human, recombinant (1st WHO International Standard)	95/646[a]
Placental Lactogen, Human, for immunoassay (1st International Reference Preparation)	73/545
Proinsulin, Human, for immunoassay (1st International Reference Reagent)	84/611
Proinsulin, Porcine, for immunoassay (1st International Reference Reagent)	84/528
Prolactin Human, recombinant, glycosylated (1st WHO Reference Reagent)	98/580[a]
Prolactin, Human (3rd WHO International Standard)	84/500
Prolactin, Human, recombinant (1st WHO Reference Reagent)	97/714
Prolactin, Human, recombinant, non-glycosylated (1st WHO Reference Reagent)	98/582
Serum amyloid A protein (1st WHO International Standard)	92/680
Somatropin (2nd WHO International Standard)	98/574
Tetracosactide for bioassay (1st International Reference Preparation)	80/590
Thyroid Stimulating Antibody (1st WHO International Standard)	90/672
Thyroid Stimulating Hormone, Human, for bioassay (non-WHO Reference material)	00/504
Thyroid Stimulating Hormone, Human, for immunoassay (3rd WHO International Standard)	81/565
Thyroid Stimulating Hormone, Human recombinant (1st WHO Reference Reagent)	94/674
Thyroid Stimulating Hormone, Human, recombinant, for bioassay (1st WHO International Standard)	03/192
Basic Fibroblast Growth Factor (FGF-2) rDNA (WHO 1st International Standard)	90/712
Bone − Morphogenetic Protein-2 rDNA (WHO Reference Reagent)	93/574
Brain-derived neurotrophic factor (BDNF) rDNA (WHO Reference Reagent)	96/534
Ciliary Neurotrophic Factor rDNA (WHO Reference Reagent)	94/684
Epidermal Growth Factor rDNA (WHO 1st International Standard)	91/530
Epidermal Growth Factor (1−52) rDNA (WHO 1st International Standard)	91/550
Fms-like tyrosine Kinase-3 rDNA (WHO Reference Reagent)	96/532
Granulocyte Colony Stimulating Factor rDNA (WHO 2nd International Standard)	09/136[a]
Granulocyte Macrophage Colony Stimulating Factor rDNA (WHO 1st International Standard)	88/646[a]
Hepatocyte Growth Factor/Scatter Factor rDNA (WHO 1st International Standard)	96/564
Hepatocyte Growth Factor/Scatter Factor precursor rDNA (WHO 1st International Standard)	96/556
Interleukin-1 alpha rDNA (WHO 1st International Standard)	86/632
Interleukin-1 beta rDNA (WHO 1st International Standard)	86/680
Interleukin-1 receptor antagonist (NIBSC Reference Reagent)	92/644
Interleukin-2 Cell line derived (WHO 1st International Standard)	86/504[a]
Interleukin-2 rDNA (NIBSC Reference Reagent)	86/564
Interleukin-2 soluble receptor rDNA (NIBSC Reference Reagent)	97/600
Interleukin-3 rDNA (WHO 1st International Standard)	91/510
Interleukin-4 rDNA (WHO 1st International Standard)	88/656
Interleukin-5 rDNA (WHO Reference Reagent)	90/586
Interleukin-6 rDNA (WHO 1st International Standard)	89/548
Interleukin-7 rDNA (WHO Reference Reagent)	90/530
Interleukin-8 rDNA (WHO 1st International Standard)	89/520
Interleukin-9 rDNA (WHO Reference Reagent)	91/678
Interleukin-10 rDNA (WHO Reference Reagent)	93/722
Interleukin-11 rDNA (WHO Reference Reagent)	92/788[a]
Interleukin-12 rDNA (WHO Reference Reagent)	95/544
Interleukin-13 rDNA (WHO Reference Reagent)	94/622
Interleukin-15 rDNA (WHO Reference Reagent)	95/554
Interleukin-17 rDNA (WHO Reference Reagent)	01/420
Interleukin-18 rDNA (WHO Reference Reagent)	03/200
Interferon alpha leukocyte (WHO 1st International Standard)	94/784
Interferon alpha 1 rDNA (WHO 1st International Standard)	83/514
Interferon alpha 1/8 rDNA (WHO 1st International Standard)	95/572
Interferon alpha 2a rDNA (WHO 2nd International Standard)	95/650[a]
Interferon alpha 2b rDNA (WHO 2nd International Standard)	95/566[a]
Interferon alpha 2c rDNA (WHO 1st International Standard)	95/580
Interferon alpha n1 lymphoblastoid (WHO 2nd International Standard)	95/568

Table 2 (*continued*)

International Standards/Reference Reagents	Code
Interferon alpha n3 leucocyte (WHO 1st International Standard)	95/574
Interferon alpha consensus rDNA (WHO 1st International Standard)	94/786
Interferon omega rDNA (WHO 1st International Standard)	94/754
Interferon beta rDNA (WHO 3rd International Standard)	00/572[a]
Interferon beta Ser17 rDNA (NIBSC Reference Reagent)	00/574[a]
Interferon beta, Fibroblast (NIBSC Reference Reagent)	00/576
Interferon gamma rDNA (NIBSC Reference Reagent)	87/586
Interferon gamma, Human Leukocyte (British Working Standard)	82/587
Keratinocyte Growth Factor (FGF-7) rDNA (WHO Reference Reagent)	03/150
Keratinocyte Growth Factor (24–163) rDNA (WHO Reference Reagent)	03/148
Leptin rDNA (WHO 1st International Standard)	97/594
Leukemia inhibitory factor rDNA (WHO Reference Reagent)	93/562
Macrophage colony stimulating factor rDNA (WHO 1st International Standard)	89/512
Nerve Growth Factor rDNA (WHO Reference Reagent)	95/566
Neurotrophin rDNA (NIBSC Reference Reagent)	98/718
Oncostatin M rDNA (WHO Reference Reagent)	93/564
Platelet derived growth factor BB rDNA (WHO 1st International Standard)	94/728
Stem Cell Factor rDNA (WHO Reference Reagent)	91/682
Thrombopoietin rDNA (NIBSC Reference Reagent)	03/124
Transforming Growth factor beta-1 rDNA (NIBSC Reference Reagent)	89/514
Transforming Growth factor beta-2 rDNA (NIBSC Reference Reagent)	90/696
Transforming Growth factor beta-3 rDNA (NIBSC Reference Reagent)	98/608
Tumor necrosis factor-alpha rDNA (WHO 2nd International Standard)	88/786
Tumor necrosis factor-beta rDNA (WHO Reference Reagent)	87/640
Tumor-necrosis-factor related apoptosis inducing ligand rDNA (WHO Reference Reagent)	04/166
Tumor necrosis factor soluble receptor I rDNA (NIBSC Reference Reagent)	96/528
Tumor necrosis factor soluble receptor II rDNA (NIBSC Reference Reagent)	93/524
Vascular Endothelial Growth Factor 165 rDNA (WHO Reference Reagent)	02/286

[a] Denotes standards available for biosimilars (approved or in development).

normally expressed as a statement of the statistical confidence limits of the potency estimate. Bioassays must be carefully designed to minimize and allow an assessment of the errors associated with the derived potency value.

4. Inappropriate uses of Reference Products and WHO International Standards/Reference Reagents

Reference products are, in effect, approved clinical products usually obtained in the containers in which they are marketed. They normally have a nominal content of biological activity, with specifications around this for acceptance. For example, a product may have a nominal potency of 100 units/ml with acceptance limits of 80–125% around this. Thus, they do not have a single defined value of biological activity. In the example quoted, the actual biological potency could be any value between 80 and 125 units/ml and there is no way of knowing what this value is. This implies that they cannot be used to calibrate assays. Reference standards, such as WHO international standards have a defined unitage and so they are ideally suited for the calibration of assays.

WHO international standards/reference reagents are not clinical products, even though the active substance in them may be derived from a product that was produced at clinical grade. They do not have any history of clinical use and are often formulated using excipients which are very different to those used in clinical products. They are usually lyophilized during their preparation, which means they have been subjected to additional procedures than those used for clinical products. Therefore, these reagents are clearly inappropriate for use as comparators for biosimilar product development and should not be used for such purposes. Reference products are essential for demonstrating the similarity of the SBP.

5. Conclusions

For the development of SBPs, the use of a reference product for the comparability studies is essential. There has been some discussion on providing 'standard' reference products which can be used for in the development of SBPs, but this is not feasible as the reference product must be a commercial clinical product with an established clinical record. Reference products cannot be used as 'standards' for calibration of assays.

In contrast, WHO international standards and reference reagents are used for calibrating procedures, particularly bioassays. They are not clinical products, and therefore cannot be used as comparators during SBP development.

Conflict of interest

The authors have disclosed no potential conflicts of interests.

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